

Pest Management Grants Final Report

Title: Rice Water Weevil Management: Development of Improved Sampling Techniques and Rice Yield Loss Understanding for Optimizing IPM in California Rice

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The assistance of Richard Lewis, Emily Blanco, and the UC-Davis Entomology Rice Lab was appreciated. The cooperation of the Rice Experiment Station and the staff greatly facilitated this work.

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EXECUTIVE SUMMARY

The switch from applications of preventative, preflood, granular insecticides to foliar, post-flood materials for rice water weevil (RWW) control in California rice has opened up opportunities for refined IPM in this system. However, applied research is needed to develop the information required to enhance these IPM programs and that was the goal of this research. These new insecticides were registered in 2000 as the long-time standard product was removed from the market. The use of a post-flood material may allow the inclusion of field sampling and decision guides before insecticide application. An aquatic barrier trap has been developed in southern states rice for this use and the applicability of this trap to California conditions was evaluated in 2001 in ten rice fields. Rice production (delayed flood production in the south versus direct-seeding in California) and RWW biology (e.g., both sexes in the south and only females in California) differ significantly between the two regions, therefore the need to critically evaluate this trap under our conditions. RWW were captured in all fields in the traps and over 75% of the adults were captured by the 3-leaf stage. Given that the insecticide application must be made by the 3-4 leaf stage, this early period is critical for decision-making. There was a weak linear relationship between adult captures and the resulting larval numbers. As more adults were trapped in a field, there were more larvae in the samples a few weeks later with the relationship indicating slightly less than 1 larva per trapped adult. Another year of research in this area will solidify this relationship, but at this time the use of this trap appears fruitful. The second area of anxiety with these new insecticides is the short residual and the possibility of RWW damage after the active ingredient has dissipated. The previously used preventative insecticide provided "season-long" control. Small plots were set-up and infested with RWW adults (two different densities) at five different rice growth stages to simulate infestations after an insecticide treatment has subsided. RWW larval infestations as high as 6.5 per sample were achieved in infested plots compared with ~0.1 in the uninfested. Plant growth, development, photosynthesis, maturity, and yield were evaluated. Grain yields, the most important aspect from an economic standpoint, were reduced by rice water weevil infestations which were initiated as late as the ~8 leaf stage. This is much later than that seen in a preliminary study conducted in 2000 and later than that supported by previous observations. Year-to-year variation in environmental conditions and the resulting plant response probably account for these differences. Further definition of this area is needed.

REPORT

A. Introduction

The Rice Water Weevil (*Lissorhoptrus oryzophilus* Kuschel) is the most important insect pest of rice in California. This insect was first found in California in 1959 over a 400 sq. mile area; this relatively large infested area precluded the possibility for eradication. The California infestation apparently resulted from the introduction of one or more females from the eastern U.S. Rice is an important agricultural crop in California with about 550,000 acres per year and a total value of \$500 million per year. In the Sacramento Valley, the economies of many communities depend heavily on rice production. The poorly drained clay soils and environmental conditions in these areas limit cropping possibilities to only a few crops with rice being ideally suited. In California, rice yield losses of 10-30% from rice water weevil (RWW) infestations can commonly occur. This is the only insect that generally reaches damaging levels in California rice.

The goal of this project, and of my overall rice arthropod management program, is to develop and provide useable pest management strategies to the rice industry that are viable from an economic and entomological standpoint and are in concert with environmental and societal goals and needs. The RWW is the primary rice insect pest and my project has developed and evaluated cultural and chemical controls for this pest over the last 9 years. In recent years, we have researched alternative insecticide treatments, plant response to RWW injury, biorational insecticide efficacy, the viability of cultural control measures, insecticide application methods, RWW infestation patterns, and the susceptibility of rice varieties to RWW.

The rice water weevil overwinters as an adult in a diapause state. The overwintering sites include on levees and ditch banks, in the crop residue in the basins, in riparian areas, etc. As the temperatures increase, the adults feed on leaves of grasses and eventually break the diapause. The adults can fly for several miles (hypothesized to be up to 20 miles). The spring flight (April to June) occurs during days characterized by warm, calm evenings. During these periods, the adults fly and prefer to infest newly flooded rice fields. The adults feed on the leaves of rice plants, which result in characteristic longitudinal feeding scars. This feeding has no effects on rice growth or yield; however, coinciding with this the adults oviposit in the rice leaf sheaths found just below the water level. This oviposition generally occurs in plants with from 2 to 6 leaves, but during some years can be more prolonged. Eggs hatch in 5-7 days; the first instar larvae feed on the leaf tissue for a few days and then drop down through the water and soil to the roots. The remaining portion of the life cycle is spent in the flooded soil of rice fields. The larvae develop through four instars and feed on rice roots doing significant damage. Pupation occurs on the rice roots and the new adults emerge in late July. These adults feed to a limited extent on rice leaves and then leave the rice fields for overwintering sites.

Management of rice water weevil in California relies on chemical and cultural controls. Biological control of this pest is nonexistent. The adult weevils infest rice fields a few days after flooding and before the establishment of a plant canopy or the aquatic arthropod community. The larval and pupal stages are in the flooded soils and protected from the activity of most arthropods. Some moderate host plant resistance has been identified to RWW and is being

incorporated into commercial varieties, but this does not appear to be a stand-alone management tool. Cultural controls are of some utility for management of rice water weevil in California. Removal of levee vegetation in the spring helps reduce RWW densities in the adjacent rice basins. The additional herbicides required for this and the loss of wildlife habitat on the levees are substantial drawbacks of this management technique. Two additional cultural methods assist in reducing rice water weevil densities, but inherently result in lower rice yields. There include dry seeding rice and delayed seeding dates. The reduced yields result in these techniques being unacceptable to growers. Preliminary research by my laboratory has shown that winter-flooding reduces RWW populations the following spring, but research has not progressed to the point where sound recommendations can be given to growers.

Chemical control of RWW has relied on carbofuran (Furadan® 5G) since the late 1970's. Until 1999, this had been the only insecticide registered for RWW management. Carbofuran was used in California on about 35-40% of the rice acreage; average usage in 1994-97 was about 58,000 pounds active ingredient each year with about 2,900 applications. The granular insecticide was applied before flooding and incorporated into the soil. This ~35-40% usage represents a much higher number of fields because most growers applied carbofuran to the first ~30 feet of the basin nearest the levee. The higher rice water weevil densities nearer the levees result in this being the only area where management tools are needed. Furadan 5G registration was cancelled after the 2000 season.

In 1999, two new insecticides were registered as alternatives to Furadan. In our studies, these insecticides showed that they can provide effective RWW management in California; however, they have some limitations and will require considerable changes in rice production practices. These two insecticides are diflubenzuron (Dimilin®) and lambda-cyhalothrin (Warrior®). The most important change is that diflubenzuron and lambda-cyhalothrin have to be applied after flooding and seedling emergence; these insecticides have no effects on RWW larvae, which is the damaging stage. They manage this pest by targeting the adults and minimizing the deposition of viable rice water weevil eggs. Dimilin sterilizes the RWW adults (i.e., females produce no viable eggs) and Warrior kills the adults. Application timing is of utmost importance since no control is possible with these products after a few days following oviposition. These insecticides are recommended to be sprayed at the 3-5 rice leaf stage.

The post-flood application nature of these new insecticides may allow the rice growers to assess the infestation severity of rice water weevils before taking any other action. Presently, California rice growers do not have a sampling technique that evaluates the level of adult infestation in the field, and therefore, they use these insecticides as a prophylactic measurement, i.e., spray the fields beginning at the 3-5 leaf stage. The present threshold of 10 to 20 % scarred plants was designed for post-flood Furadan applications. With this level of scarring, a significant number of rice water weevil eggs would already be deposited and thus this threshold is not applicable to Dimilin or Warrior. In Arkansas, Hix and his collaborators, have developed a floating barrier trap for RWW adults as a sampling tool. This study was designed to evaluate the applicability of this trap as a sampling tool in California rice.

The second consideration following the registration of the post-flood insecticides in 1999 is the possibility that more than one application may be needed to optimize control. These two active ingredients are short-lived in the water (7-10 days), which is an advantage in the sensitive aquatic environment but a disadvantage when trying to provide residual RWW control. Central to this question of multiple applications is the need for data on rice plant response to RWW injury at various points in the growing season. RWW infestations begin at the ~3 leaf stage (concurrent with the recommended application timing) but later infestations can also occur. The second objective dealt with this question.

Two of the DPR priority areas will be addressed through this proposal, 1.) alternatives for carbamate registrations and 2.) protection of surface and ground water quality. Two specific objectives were addressed as follows:

Objective 1.) *Investigate the existing monitoring protocols for determining the need for Rice Water Weevil treatment, and refine/develop additional monitoring techniques that may be useful for determining the need for treatment*

The following tasks were outlined to make progress towards this objective: 1.) select grower cooperators for studies, 2.) fabricate traps designed to capture a representative number of RWW adults, 3.) place floating traps in rice fields within 2 days after rice seeding, 4.) monitor traps every 2 days and collect captured RWW adults, 5.) record field conditions, 6.) evaluate RWW larval populations, 7.) data analyses, 8.) reporting data to grower cooperators and appropriate reports.

Objective 2.) *Evaluate the relationship between Rice Water Weevil induced injury and rice yield at various plant growth stages so as to determine the length of time that RWW control is warranted.*

The following tasks were outlined to make progress towards this objective: 1.) select field site for study, 2.) set up aluminum rings for the plots, 3.) seed rice into plots, 4.) cover rings with row cover, 5.) infest rings with RWW adults, 6.) establish a consistent plant population, 7.) evaluate RWW feeding scars, 8.) evaluate RWW larval populations, 9.) evaluate rice grain yields, 10.) data analyses, 11.) reporting data to grower cooperators and appropriate reports.

B. Materials and Methods

Objective 1:

We obtained cooperation from 8 growers, and along with the Rice Experiment Station and straw management sites (the latter on grower property but managed by UC for this project); therefore we had 10 sites in total. Grower cooperation involved allowing us to place traps in their rice basins and not treating those basins with insecticides. Fields in Butte, Colusa, Sutter, and Glenn counties were used for testing. A light trap was operated at the Rice Experiment Station in order to determine the flight patterns for RWW in 2001. This allowed us to determine how timing of rice seeding compared with the timing of RWW flight. We fabricated ~100 floating barrier traps following the Arkansas model (Hix, R.L., D. T. Johnson, J. L. Bernhardt. 2000. *An aquatic barrier trap for monitoring adult rice water weevils (Coleoptera: Curculionidae)*. *Fla. Entomol.* 83: 189-192). Our plan for 2001 was to investigate the utility of this trap. Going into this season, we recognized two potential problems 1.) algal contamination

of trap and 2.) low water level due to field draining. We didn't attempt to address these problems in 2001, but rather to see if this trapping method has promise. We hand-cleaned the traps and measured water depth to minimize and adjust, respectively, for these issues. If promise was shown, than some measures to refine the trap may be needed in further years. Traps were placed into each field within 2 days of flooding/seeding. The average date of placement into the field was 15 May. Eight traps were placed into each field. Traps were placed about 10-15 feet from the field edge (area of highest RWW populations) and at least 50 feet apart. Traps were monitored three times per week. This involved removing captured RWW adults from each of the two collection cups per trap, cleaning the algae from the screens, and collecting the field data as detailed below. Traps were operated for 11 to 21 days depending on the field and the rice growth. Rice leaf stage, water depth, and trap condition were recorded for each trap on each sample date. Leaf stage averaged 1 leaf (first leaf just "spiking") and 7 leaves at the time of trap placement and trap removal, respectively. RWW immatures were sampled twice from nine of the ten fields (one location was drained for an extended period of time and by the time it was reflooded [necessary for sampling] the first sample timing was missed). The two sample timings were at about 6 and 8 weeks after seeding which corresponded with medium and large RWW larvae and large RWW larvae and pupae, respectively. In each field on each sample date, 40 core samples were collected, taken to the laboratory, and processed to separate RWW immatures from the soil.

Objective 2:

A field site was obtained at the Rice Experiment Station for this study. Seventy-two rings were set-up on 8 May and seeded with 'M-202' on 18 May. The amount of seed for each ring had been previously weighed, soaked for 24 hours, and drained for 24 hours. Sixty-eight rings were covered with floating row cover on 18 May; one set of rings, i.e., one in each of the four blocks, was left uncovered to ascertain the effects of the row covering on rice plant growth and development. RWW adults were collected from nearby untreated grower fields and placed into the covered rings. The infestations started on 25 May when the rice was in the 2 leaf stage. Later infestations were done on 1 June, 8 June, 15 June and 22 June (~9 leaf stage). On each date, two infestation intensities were used (0.3 and 0.6 RWW adults per rice plant) and there were four blocks of each treatment arranged in a randomized complete block design. Previous studies have shown the larvae resulting from these infestation levels will cause moderate and severe stress, respectively, to the rice plants. A total of 1740 RWW adults were utilized. Plant populations of 12 plants per sq. ft. were established on 5 June. By this date, all seeds had germinated and established. Plants were counted and hand manipulated in each ring. Extra plants from rings were transplanted into rings that were deficient in plants. By controlling plant populations and other factors, we can examine only the influence of RWW feeding.

Plants were visually evaluated for RWW feeding scars on 1 June, 8 June, 15 June, 22 June, and 29 June. Core samples (44 in³) were collected on 29 June, 12 July, 27 July, and 10 Aug.; RWW immatures were recovered with a washing-flotation technique. Several plant response variables, plant height, number of tillers, dry leaf weight, root length, dry root weight, and leaf area, were examined on each date. Concurrent with this, rice plant physiological, photosynthesis, stomatal conductance, etc., parameters were quantified. A portable photosynthesis instrument was carefully (since it is not waterproof) floated in the plot area and utilized. Rice plant maturity, quantified with panicle emergence, was recorded frequently in

August in all treatments. Rice yield were quantified in mid-October. The number of panicles per ring was counted. The above-ground biomass was clipped and weighed as a measure of total growth and this material was threshed and the grain recovered. Grain weight and percentage moisture were determined on the clean grain. The weight of 200 kernels was determined and average kernel weight calculated. The relationship between rice grain yield at 14% moisture and RWW larval densities was examined.

C. Results

Objective 1

RWW were captured in all fields; a total of 418 RWW was captured. It was surprising the number of adults that were captured when the rice was in the 1-leaf stage, i.e., just starting to germinate (Fig. 1). Over 50% of the adults were captured during this time, which was generally during the first 5 days after seeding. By the 3-leaf stage, over 75% of the RWW had been captured. This bodes well for the usefulness of the trap as this period corresponds to when management decisions must be made, but these results do differ from those from Arkansas where the trap was developed. The majority of rice field seeding (at least for the fields used in this study) occurred after the peak in RWW flight, i.e., early May. RWW captures in the barrier traps were generally higher when the time of seeding was near the timing of peak flight as indicated by the light trap (Fig. 2). Water depth ranged from 0 (drained) to 13 inches during the period of trapping. There was a poor relationship between water depth and rice leaf stage (Fig. 3). Trap conditions ranged from good, i.e., floating well, clean of algae and sediment to poor, i.e., listing due to low water, screening material covered with algae. Overall the trap condition was acceptable.

The results showed a relationship between adult captures and the resulting larval numbers (Fig. 4). The relationship was a weak linear relationship between these two parameters. As more adults were trapped in a field, there were more larvae in the samples a few weeks later; however, there was considerable variability in the data. In 2001, for every adult captured in a trap, slightly less than 1 larva resulted. Another year of research in this area should add some verification to these data.

Objective 2

RWW adults were placed in the ring plots as planned. Details for the treatments and dates are shown in Table 1. Results from adult scarring evaluations closely reflected the infestation regimes. The uninfested, covered plants had little to no feeding, i.e., no RWW adults “squeezed” through the covers (Fig. 5). The uninfested, uncovered rings had slight feeding (0 to 10% scarred plants). The 0.6 RWW/plant infestation regime had nearly 100% scarred plants and the 0.3 RWW/plant was similar. These treatments differentiated slightly, i.e., the 0.3 showed less damage than the 0.6, two or more weeks after infestation and in the infestations on larger plants. Very few larvae were found in the treatments that were not hand-infested (Fig. 6). In some years with similar studies, the row cover was needed to exclude a natural infestation, but in 2001 the RWW flight and potential for a natural infestation was apparently past by the time the rings were set-up. With the infestations during the younger plant growth stages, there was not a noticeable separation in larval numbers between the 0.3 and 0.6 infestations. This is not surprising because the larval survival is often limited by root availability. With the later infestations, the 0.6 infestation had significantly more larvae than the 0.3 infestation. It is

anticipated that different yield response curves will be seen with the range of infestation timings.

RWW infestation reduced plant height (Fig. 7), plant dry weight biomass (Fig. 8), and the number of tillers per plant (Fig. 9). The effects were greatest with infestation at the early plant growth stages (2 leaf) vs. later stages, i.e., 8 leaf. For example, plant height was reduced by 24% by the '0.6 RWW at the 2 leaf stage' treatment during the time of peak larval density and 6% by the '0.6 RWW at the 2 leaf +28 day' treatment during the time of peak larval density. There was little recovery in plant height from early damage (height differential between infested and uninfested remained). Plant dry weight was influenced significantly by infestation density and timing. The most severe infestation (0.6 RWW at the 2 leaf stage) reduced weights by over 50% on 13 Aug. Less severe treatments showed no decrease in plant biomass compared with the uninfested. Similar results were seen with tillering. Early and high RWW density treatments reduced tillering by up to 50%.

Panicle emergence started on ~7 August 2001. Rice development was influenced by the severe and early RWW infestation and resulting injury. The 0.6 RWW infestation at the 2 leaf stage delayed panicle emergence by 5-7 days. The other treatments had no effects (only few shown for simplicity) and they reached 90% panicle emergence by ~mid-August (Fig. 10). Results from previous years showed no effect of RWW injury of plant maturity.

Grain yield losses from RWW were more persistent than that indicated from other studies and from observations. This depicts the year-to-year variation in plant response to insect feeding. The treatments that were not infested with RWW averaged about 6500 lbs./A grain. There were significant linear relationships depicting grain loss with the '2 leaf + 7 days', '2 leaf + 14 days', '2 leaf + 21 days', and '2 leaf + 28 days' infestations (Fig. 11). Plots infested at the 2 leaf stage did not have a significant linear relationship between larval numbers and grain yield; however, the larval population in this treatment was only moderate (not enough root tissue to support larval population). Grain yields were reduced by 3.5, 7.1, 3.9, and 5.8% per larva as the infestations progressed from 7 to 28 days after 2 leaf timings. The most severe yield losses arose from reduced number of panicles (up to 41.8%) and several of the treatments reduced the panicle counts by 20-25%. Average kernel weight was largely unaffected by the insect-induced stress.

D. Discussion

Objective 1

This sampling tool shows promise. Traps were evaluated under a range of conditions in 10 rice fields in 2001. A total of 80 traps were utilized. We evaluated adult capture five to nine times per field. The data were overall positive, but this is certainly not a finished product yet. These traps are relatively easy to assemble (although a commercial source may soon be available). They are easy to place in the field and we had no problems in this area. Algae and low water level are complications and we hope to set-up some small plot studies to quantify the influence of these factors. Awareness has been created within the rice community about this work and I believe they would accept this technology.

The barrier trap, i.e., potential reduced risk tool, is not yet ready to demonstrate on a larger scale. We are still in the stage of investigating if it has a place in rice IPM in California. If it is proven cost-effective, I see no reason why growers will not adopt it. The Univ. of Arkansas

is pursuing a commercial entity to market these traps. Growers continue to question, on a field-to-field basis, the need for a post-flood insecticide application for RWW and that is the exact question this research and trap addresses. During the field days in the late summer and winter UC rice production growers showed interest in this tool.

Objective 2

This objective was successfully addressed in 2001. Again, growers show interest in the data because the study addresses a question of interest to them. With the surprising result in 2001, the question is far from being answered.

E. Summary

The switch from applications of preventative, preflood, granular insecticides to foliar, post-flood materials for rice water weevil (RWW) control in California rice has opened up opportunities for refined IPM in this system. However, applied research is needed to develop the information required to enhance these IPM programs and that was the goal of this research. These new insecticides were registered in 2000 as the long-time standard product was removed from the market. The use of a post-flood material may allow the inclusion of field sampling and decision guides before insecticide application. An aquatic barrier trap has been developed in southern states rice for this use and the applicability of this trap to California conditions was evaluated in 2001 in ten rice fields. Rice production (delayed flood production in the south versus direct-seeding in California) and RWW biology (e.g., both sexes in the south and only females in California) differ significantly between the two regions, therefore the need to critically evaluate this trap under our conditions. RWW were captured in all fields in the traps and over 75% of the adults were captured by the 3-leaf stage. Given that the insecticide application must be made by the 3-4 leaf stage, this early period is critical for decision-making. There was a weak linear relationship between adult captures and the resulting larval numbers. As more adults were trapped in a field, there were more larvae in the samples a few weeks later with the relationship indicating slightly less than 1 larva per trapped adult. Another year of research in this area will solidify this relationship, but at this time the use of this trap appears fruitful. The second area of anxiety with these new insecticides is the short residual and the possibility of RWW damage after the active ingredient has dissipated. The previously used preventative insecticide provided "season-long" control. Small plots were set-up and infested with RWW adults (two different densities) at five different rice growth stages to simulate infestations after an insecticide treatment has subsided. RWW larval infestations as high as 6.5 per sample were achieved in infested plots compared with ~0.1 in the uninfested. Plant growth, development, photosynthesis, maturity, and yield were evaluated. Grain yields, the most important aspect from an economic standpoint, were reduced by rice water weevil infestations which were initiated as late as the ~8 leaf stage. This is much later than that seen in a preliminary study conducted in 2000 and later than that supported by previous observations. Year-to-year variation in environmental conditions and the resulting plant response probably account for these differences. Further definition of this area is needed.

APPENDICES

Publications

Godfrey, L. D., R. R. Lewis, and C. J. Yip. 2001. Status report on post-flood rice water weevil control tools - year 1. Oral presentation and abstract (p. 33-36) at the Rice Experiment Station Field Day, Aug. 29, 2001.

Godfrey, L. D., R. R. Lewis, E. Blanco. 2001. Can in-field monitoring be used to improve rice water weevil management? Poster presentation and abstract (p. 19-20) at the Rice Experiment Station Field Day, Aug. 29, 2001.

Godfrey, L. D., and R. R. Lewis. 2002. Rice Plant Tolerance to Rice Water Weevil Induced Injury Increases with Plant Age. Rice Technical Working Group, Feb. 2002; poster and submitted abstract.

Lewis, R. R. and L. D. Godfrey. 2002. Use of Arkansas Designed Floating Trap to Aid Decision Making for Post Flood Insecticide Applications in California Rice. Rice Technical Working Group, Feb. 2002; poster and submitted abstract.

Table 1. Details for treatments for Objective 2.

<u>Factor 1</u>			<u>Factor 2</u>
Timing	Plant Growth Stage	Date	RWW Infestation Severity
2 leaf stage	2 leaf	25 May	1.) 0 RWW adults per plant
2 leaf stage + 7 days	3 to 3.5 leaf stage	1 June	2.) 0.3 RWW adults per plant
2 leaf stage + 14 days	5 to 5.5 leaf stage	8 June	3.) 0.6 RWW adults per plant
2 leaf stage + 21 days	7 to 7.5 leaf stage	15 June	
2 leaf stage + 28 days	9+ leaf stage	22 June	

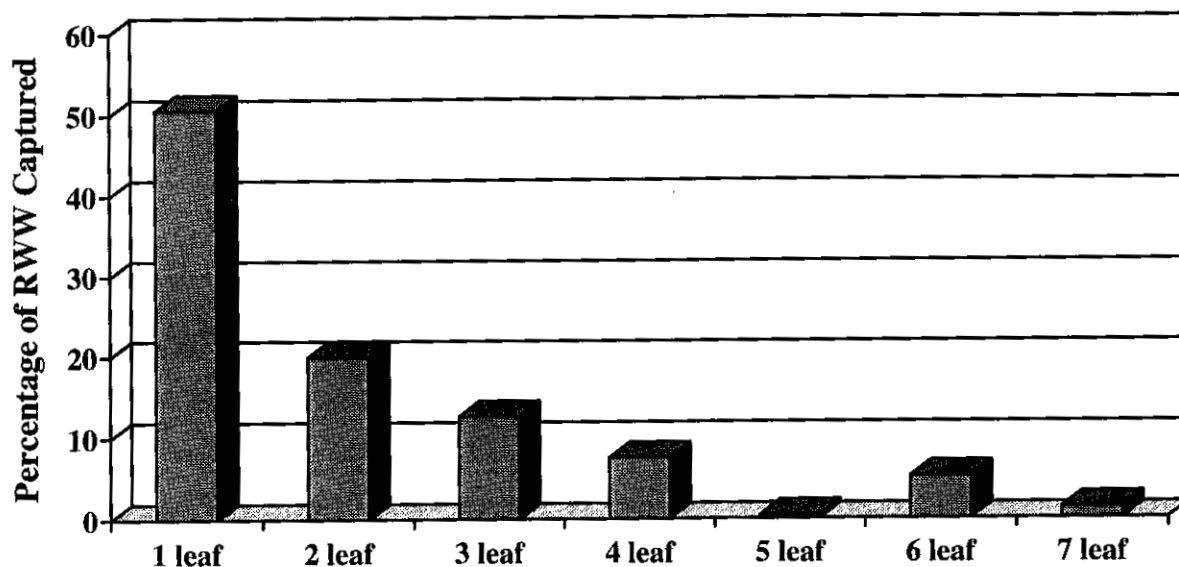


Figure 1. Percentage of RWW adults captured by floating barrier traps at various rice growth stages.

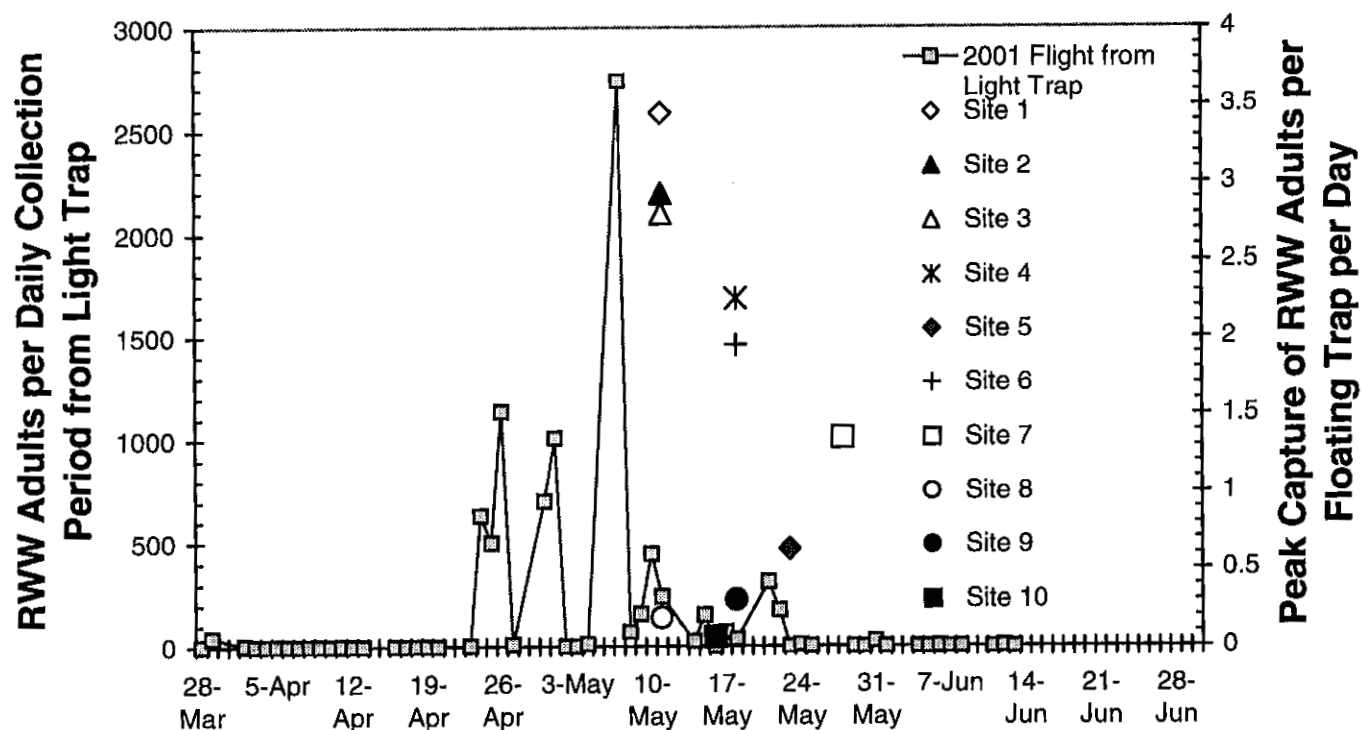


Figure 2. Relationship between RWW flight indicated with light trap and peak RWW capture in each field with barrier trap

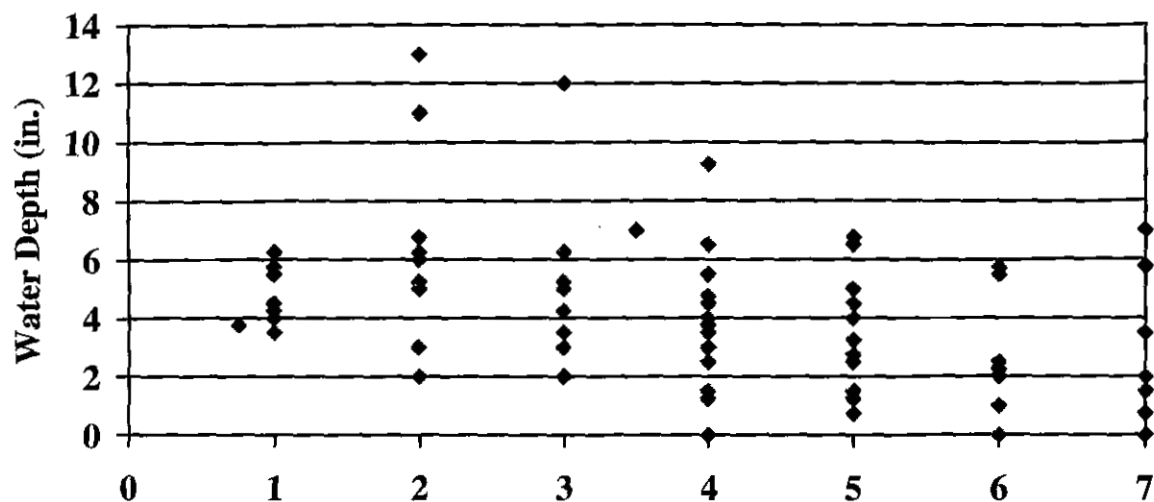


Figure 3. Relationship between water depth and rice leaf stage.

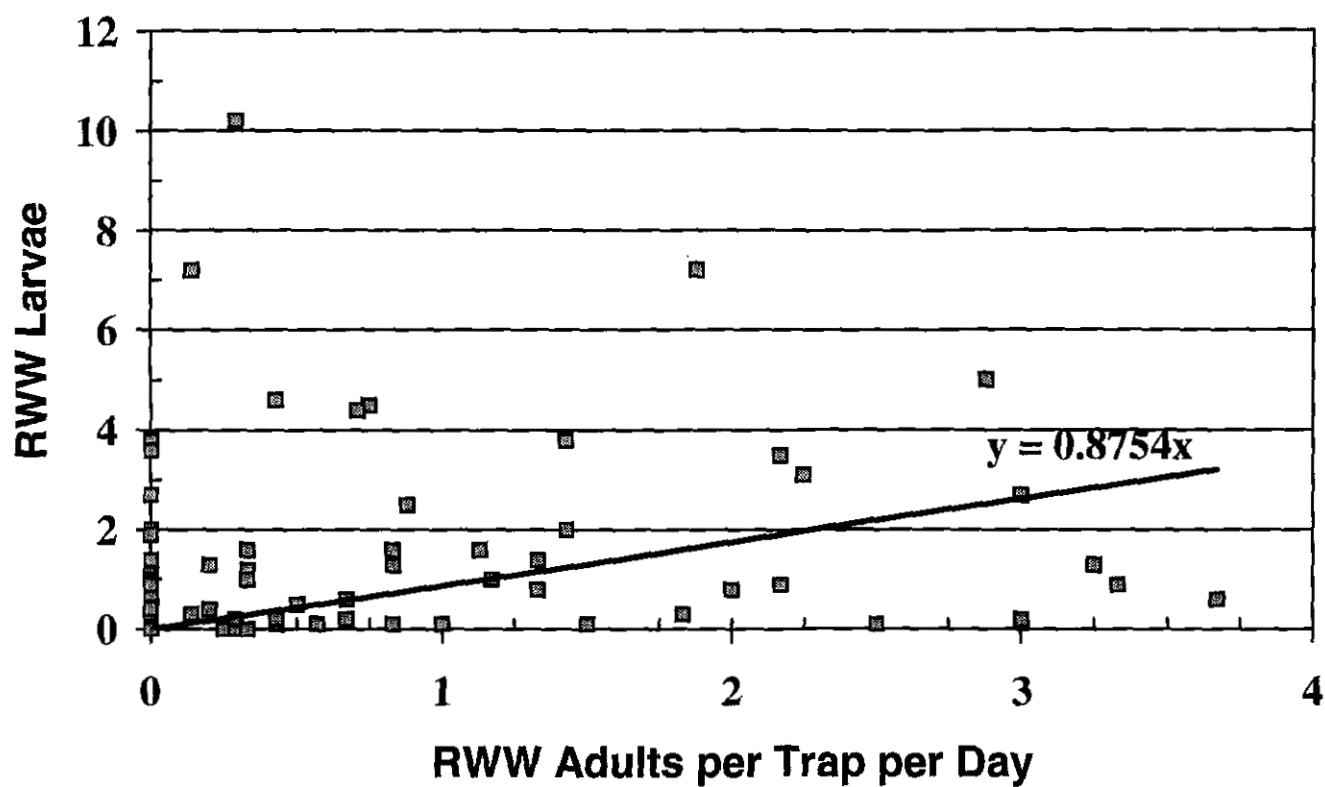


Figure 4. Relationship between RWW adult capture in floating barrier traps and RWW larval populations; summary of 10 fields, 2001.

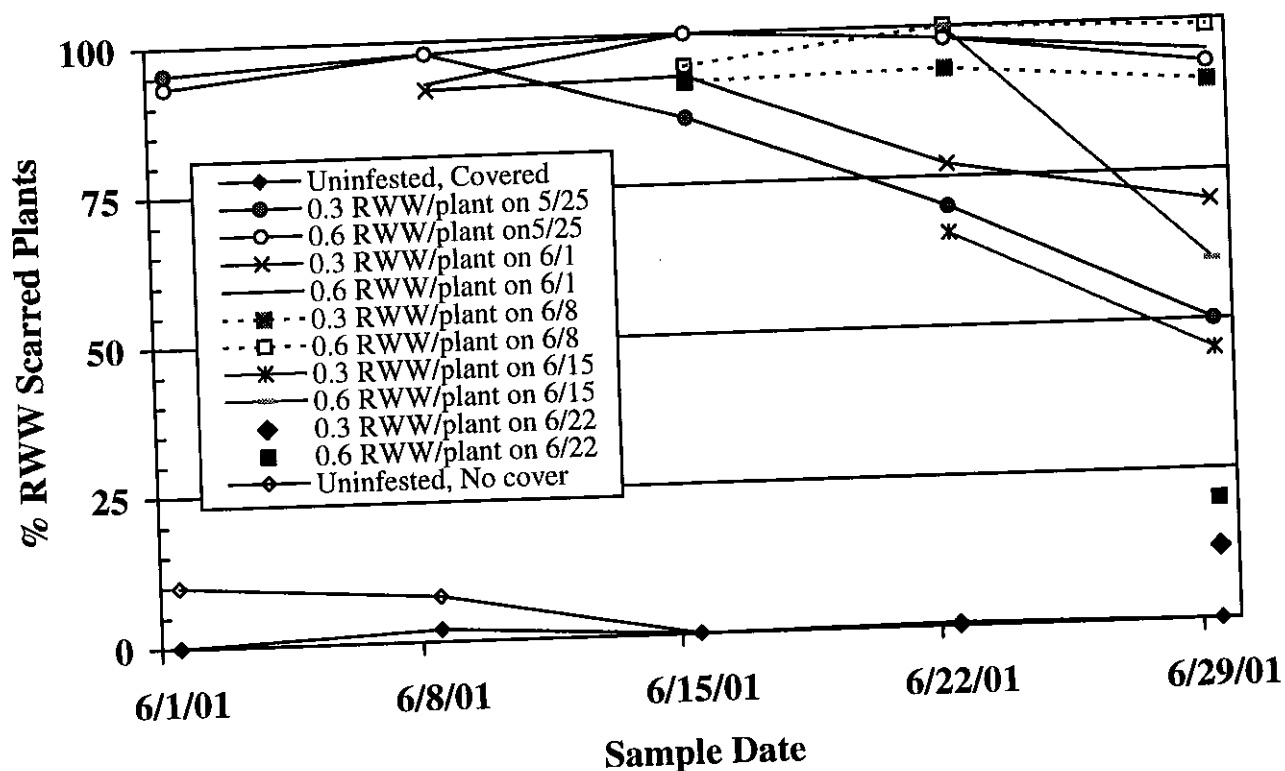


Figure 5. Percentage scarred rice plants from RWW under various infestation regimes (timing and density), 2001.

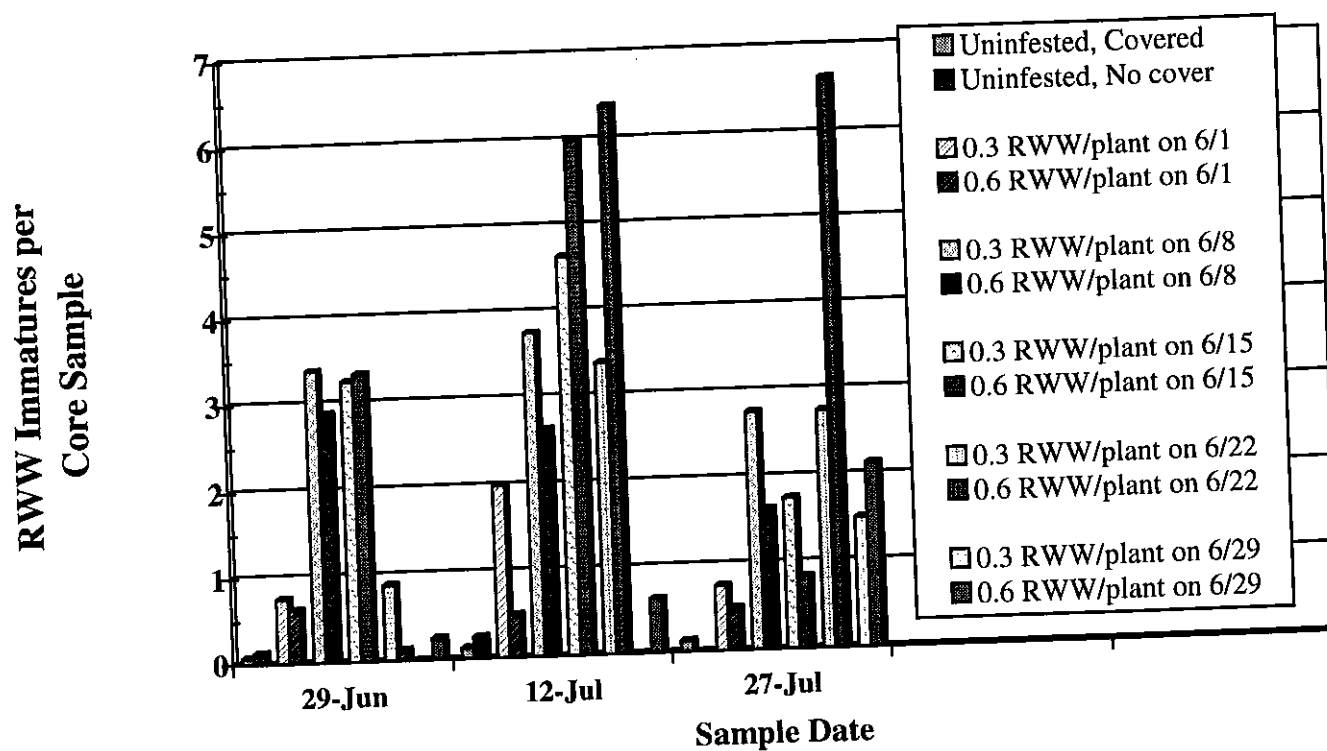


Figure 6. Populations of RWW immatures under various infestation regimes (timing and density), 2001.

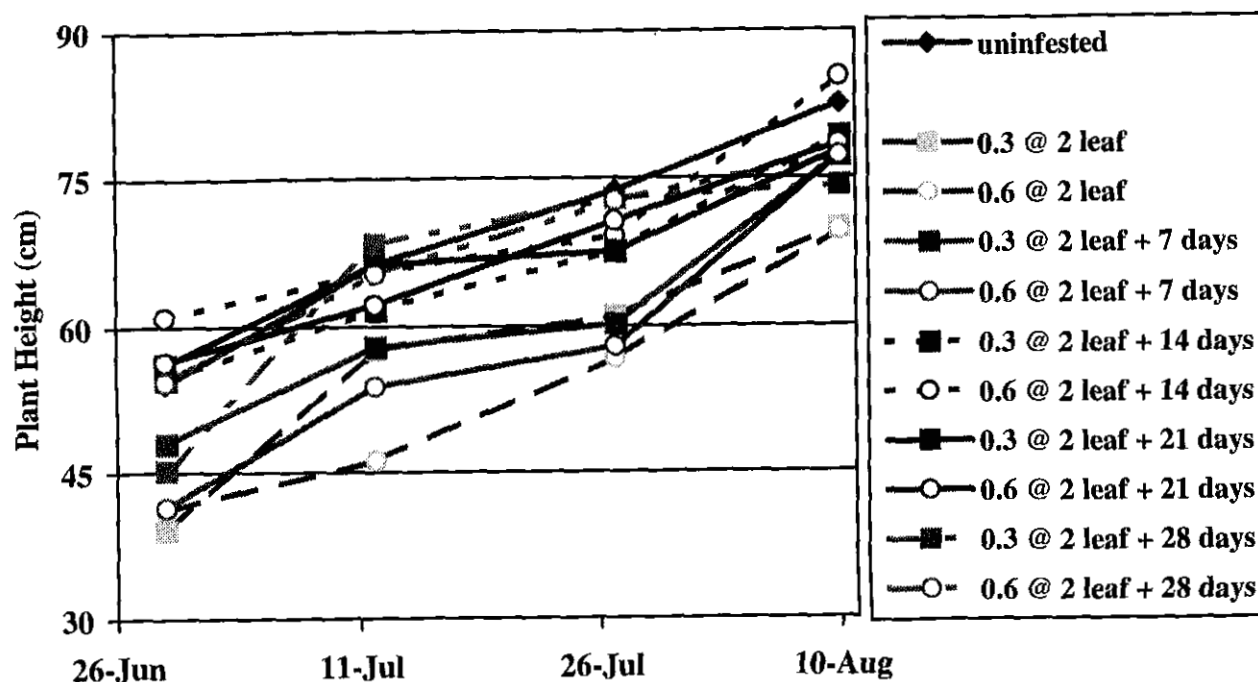


Figure 7. Influence of RWW injury (at different intensities and plant growth stages) on rice plant height.

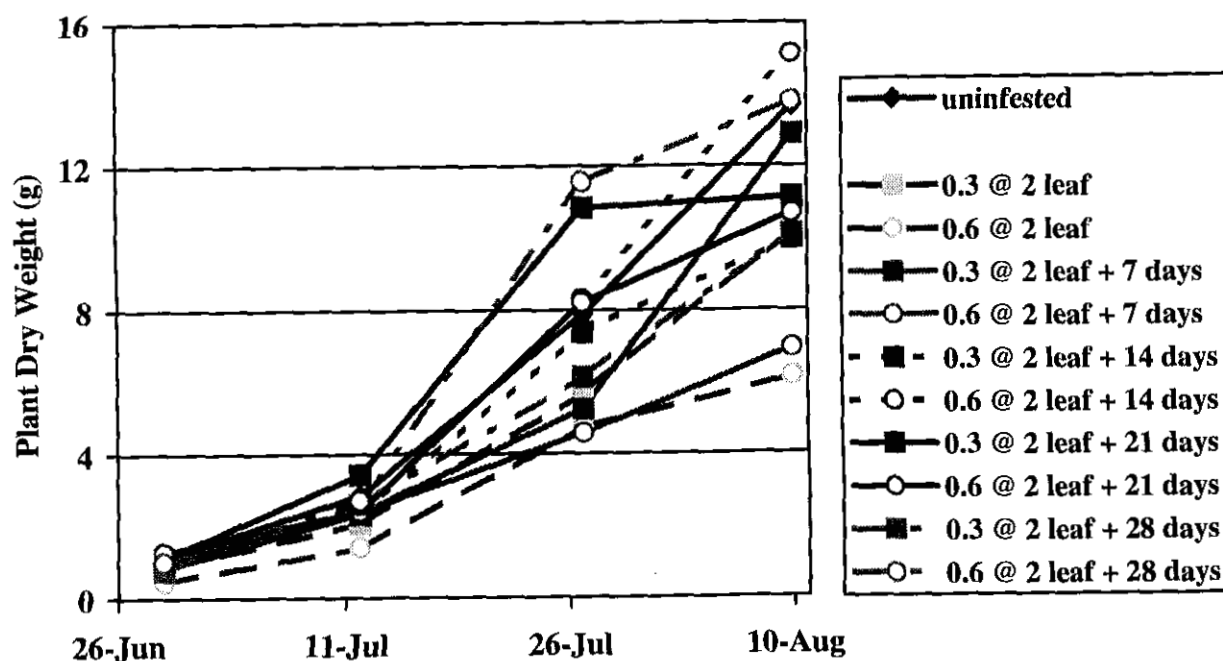


Figure 8. Influence of RWW injury (at different intensities and plant growth stages) on rice plant dry weight.

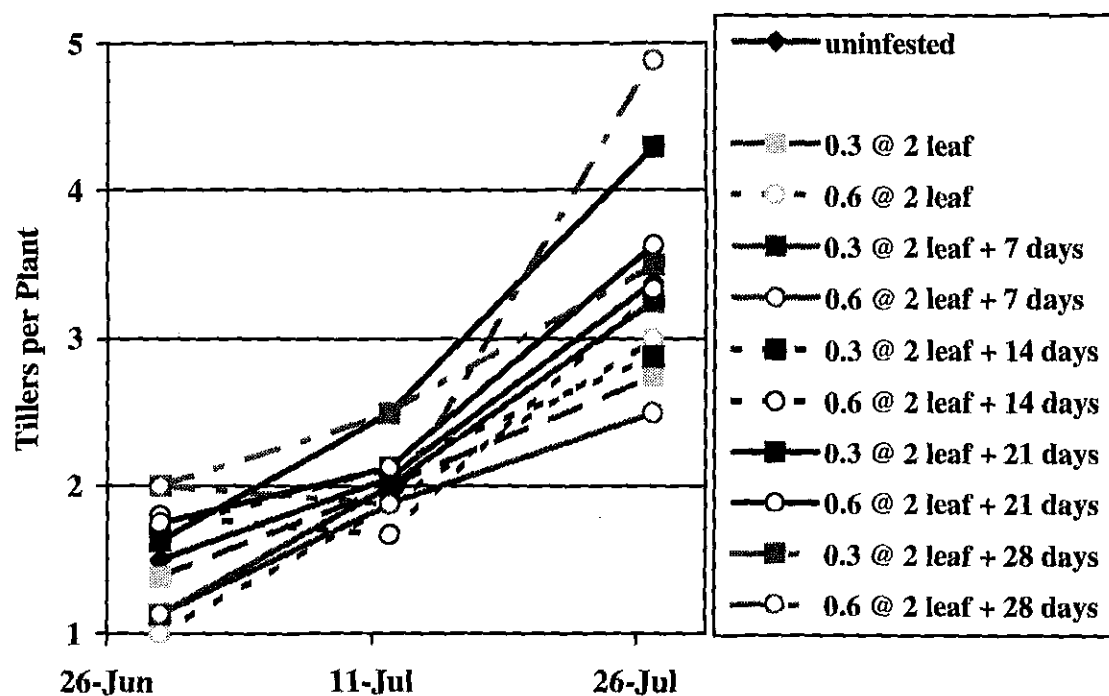


Figure 9. Influence of RWW injury (at different intensities and plant growth stages) on rice plant tillering.

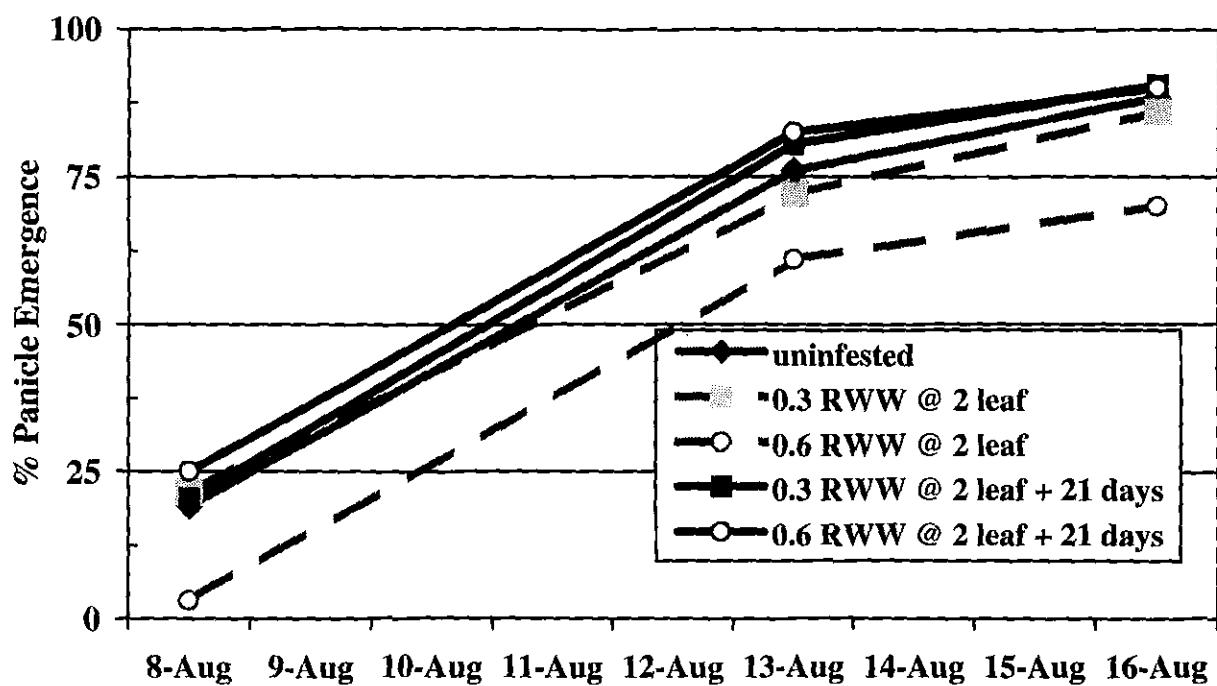


Figure 10. Influence of RWW injury (at different intensities and plant growth stages; partial list of treatments) on rice plant panicle development (maturity).

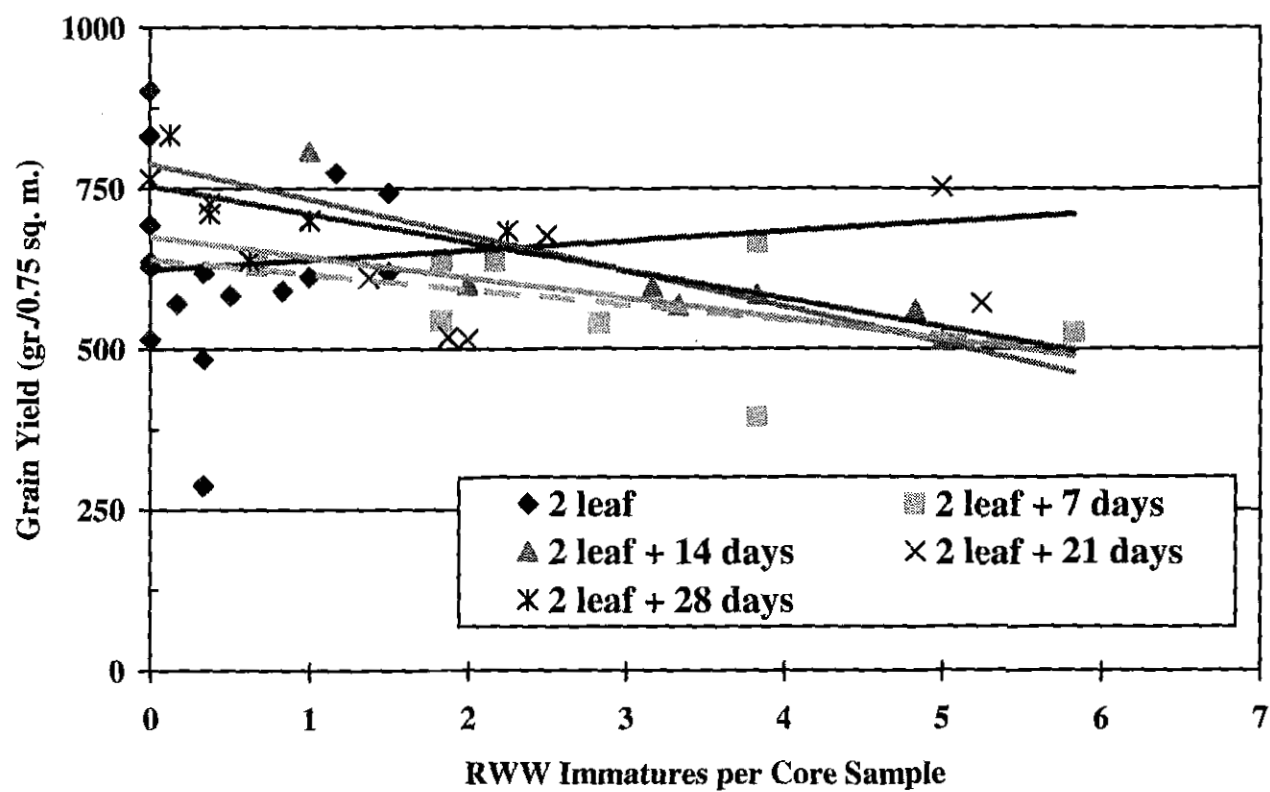


Figure 11. Relationship between RWW density and rice grain yield with infestations established at several successive plant growth stages.